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A catalogue of *Anopheles gambiae* transcripts significantly more or less expressed following a blood meal

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Abstract

The recent assembly of the *Anopheles gambiae* genome included the sequencing of ~80,000 EST from blood fed (BF) and non-blood fed (NBF) adult female mosquitoes to help find the exons and introns in the mosquito genome. These two EST libraries provided an insight into the differential gene expression resulting from the metabolically intense task of converting the massive blood meal into eggs. Previously, 168 genes have been identified to be either significantly more or significantly less transcribed after the blood meal [Science 298 (2002) 129]. Presently, 435 transcripts are described; these are grouped in supplemental tables by probable function to facilitate public access to these data and to help in designing postgenome experiments in the biology of *Anopheles gambiae*. The electronic tables and supplemental material are available at http://www.ncbi.nlm.nih.gov/projects/Mosquito/Ag-ESTs.

Keywords: Mosquito; Genome; Hematophagy; Gene expression; Gene regulation

1. Introduction

The recent publication (Holt et al., 2002; Zdobnov et al., 2002) of the *Anopheles gambiae* genome is a landmark in medical entomology, both because this was the first vector genome to be fully sequenced and because of the impact of this mosquito in public health. Combined with the genetic transformation tools that have been obtained for this insect (Grossman et al., 2001), a new era has begun—both to study the biology of this vector and its interaction with other organisms and to devise novel strategies for mosquito control.

As part of the effort to identify the open reading frames in *An. gambiae* genes, two cDNA libraries were made from adult female mosquitoes of the same age that were not blood fed and from mosquitoes that blood fed 24 h previously (Holt et al., 2002). Approximately 40,000 expressed sequence tags (EST) were sequenced from each library, which is publicly available at the National Center for Biotechnology Information (NCBI) ftp site. This library represents a snapshot of the RNA messages transcribed at each extreme of two distinct physiological states of the adult female mosquito: when it is in a metabolically less active state, and when it is converting the blood meal into eggs.

After emergence to adulthood, non-autogenous female mosquitoes such as *An. gambiae* need a blood meal to develop eggs, while most basal metabolic needs, including flight, are sustained by sugar meals. Mosquitoes search for a suitable host for a blood meal, usually at a particular time of the day or night, with the aid of vision, odor, and temperature sensors. After landing, mosquitoes insert their mouthparts into their hosts, inject saliva containing potent antihaemostatic components, and extract, in a few minutes, several times their body weight in blood. This blood meal goes to the midgut, where scouting constitutive proteases perform an initial digestion of the meal, leading to the induction, in a secretagogue mechanism, of additional proteases and other digestive enzymes. Within hours, the peritrophic matrix envelops the blood meal, creating a dialysis bag-like structure around the ingested meal. Several hormones are trig-

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gered by the blood meal, which act on the fat body and ovaries of the mosquito, switching these organs to an increased metabolic state. A highly coordinated assembly line starts: the resulting components of blood digestion—amino acids, carbohydrates, and lipids—are processed by the fat body into proteins that are subsequently taken by the ovaries into the growing oocytes. Other proteins are synthesized directly by the ovaries. In two days, the entire blood meal is converted into over 100 eggs (Clements, 1992), when the mosquito is ready to initiate another gonotrophic cycle.

Previously, a brief account of the significant differences in gene expression between blood fed (BF) and non-blood fed (NBF) *An. gambiae* was made where 168 gene products were identified as up- or down-regulated following the blood meal (Holt et al., 2002). In this report, a more detailed account of these differences is presented, where 435 transcripts are described as significantly increased or decreased following the blood meal, together with a supplemental spreadsheet where data on these genes can be easily located, in the hope that this information can accelerate the enormous task of understanding gene expression in the postgenomic age of *An. gambiae*. An interpretation of these differences, similar to that described before (Holt et al., 2002), is also provided.

2. Materials and methods

2.1. EST files

The file EST_others.Z was downloaded from the NCBI site, ftp://ftp.ncbi.nih.gov/blast/db/, and the *An. gambiae* EST extracted from the flat file by the use of script that searched the sequence definition line for the string "A.Gam.ad.cDNA." (A flat file, also known as a fasta file, has one or more sequences in which the sequence name is all contained in one line starting with a ">" followed by the sequence in the following line(s)). Accordingly, 43,174 and 39,752 EST were obtained for the BF and NBF libraries, respectively.

2.2. An. gambiae proteome spreadsheet

The *An. gambiae* proteome was obtained from NCBI Entrez (http://www.ncbi.nlm.nih.gov/Entrez/), consisting of 15,212 sequences, in October 2002; these were labeled as agCP or ebiP in their definition line. Please note that these protein sequences derive from automated annotations, and that many do not start with a methionine. Accordingly, the results and discussion indicating length of amino acid residues of these proteins should take this fact into consideration. The *An. gambiae* proteome was assembled in a hyperlinked Excel spreadsheet (Microsoft, Seattle, WA), each protein in a spreadsheet row. Comparisons were also made of each sequence to the non-redundant protein database (NR) from the NCBI, to the gene ontology database (Ashburner et al., 2000), to the conserved domains databases (CDD) of the NCBI, and to the *Drosophila melanogaster* proteome. The blast results were hyperlinked into the spreadsheet to facilitate annotation of the gene products of interest. Links to the NCBI chromosomal location for each protein gene are also provided, as well as links to the KEGG database (Kanehisa et al., 2002) when an Enzyme Commission number was available. Based on these data, a "Comments" column was manually edited to indicate the possible function of each protein.

2.3. Computational tools

Blast searches (Altschul et al., 1997) were done with the executables provided by NCBI (blastz.exe, found at ftp://ftp.ncbi.nih.gov/blast/executables). Several scripts were written in Visual Basic 6.0 (Microsoft).

2.4. Distribution of EST among the proteome set

Each EST flat file was blasted (using the program blastx) against the *An. gambiae* proteome set using a cutoff value of 1×10^{-6}. The resulting file was scanned and the best protein match, or "hit", identified. For each protein in the proteome spreadsheet, two fields recorded the number of hits each to the NFB, and to the BF libraries. EST were also compared to the *An. gambiae* mitochondrial DNA using the program blastn with a cutoff value of 1×10^{-6}.

2.5. Statistical tests

To detect statistically significant differences in the number of EST hits a protein received in each of the two libraries, the χ^2 test was used. Because both libraries have similar numbers of EST, we selected only the protein sequences that had 10 or more total hits from each library, as this would produce expected frequencies of five in each library, derived from the null hypothesis; this was done because a minimum value of five for expected frequencies is a precondition for the χ^2 test with 1 degree of freedom. Accordingly, 367 proteins were identified satisfying the above requirement. We also selected those sequences that had between six and nine total hits but for which zero was the result for either of the two libraries. The rationale was that in a series of six hits from one library and zero from the other, the first hit reflects a random "choice" for one of the two libraries, and then the following series of five hits from the same library has a $1/2^5$ chance of happening or 0.031. An additional 68 proteins were thus identified. The tables include the number of hits for critical evaluation of these results. These results contrast with the previously reported analysis (Holt et al., 2002), where using a binomial distribution and a p-value cutoff of 0.001, 97 upregulated and 71 downregulated transcripts were found in the BF group.

3. Results and discussion

Approximately 86% of EST originating from cDNA libraries of both BF and NBF mosquitoes produced highly significant matches to either the known proteome set of *An. gambiae* or to its mitochondrial genome (Table 1). This is in agreement with the recently described proteome of *An. gambiae*, estimated to be 85% complete (>1300 genes were estimated to have been missed in the first approximation of the proteome set of *An. gambiae*) (Holt et al., 2002). Of interest, the NBF group had a significantly higher proportion of expressed mitochondrial genes (13.5%) when compared with the BF (9.8%), or a 40% difference. The differences observed in Table 1 are highly significant when compared by the χ^2 test, where the largest contributions to the χ^2 score derive from the expected and observed values found in the mitochondrial gene category. If 13.5% of the BF EST were of mitochondrial origin, there should be 5785 such EST, 1576 more than found. This apparent deficit in mitochondrially expressed genes in the BF group could be due, at least in part, to the expression of genes following the blood meal, which would lead to a dilution effect of the mitochondrial gene products. The approximately 15% of EST not matching *An. gambiae* proteins could be used to identify new gene products in *An. gambiae* and will be explored elsewhere, although they may represent non-relevant products such as transposable elements or non-coding RNA, or 5′ or 3′ untranslated sequences of annotated genes. The significant differences found in observed and expected frequencies for the nuclear set of EST from BF and NBF mosquitoes (see Materials and methods), will be the object of the remainder of this work.

From the 15,212 predicted proteins in the *An. gambiae* proteome, 7580 had at least one hit with a cDNA sequence from either of the two libraries. From these, 1046 protein sequences had 10 or more hits from both libraries. From this group, the messages of 367 proteins were expressed significantly differently when the two libraries were compared. Sixty-eight sequences were further added to this group of differentially transcribed messages because they had six to nine hits in only one of the libraries, as explained in Materials and methods. A total of 435 protein sequences were thus identified as being related to messages that are significantly either increased or decreased following a blood meal. These were grouped into 12 categories suggested by the proteins they represent (Table 2).

Table 1
Distribution of EST matches according to mosquito feeding status and EST matches to nuclear or mitochondrial genome set

	Blood fed	Unfed
ESTs matching nuclear proteome set	33,200 (76.9)	28,320 (71.2)
ESTs matching mitochondrial set	4209 (9.8)	5367 (13.5)
Not matching any set	5765 (13.3)	6065 (15.2)
Total number of ESTs	43,174	39,752

3.1. Cuticular and matrix proteins (Table 3)

Fourteen messages expressing cuticular or extracellular matrix proteins appeared significantly less transcribed 24 h following the blood meal. These include reductions ranging from 1.8 to >80 times the expression level. Among these gene products are two identified by gene ontology (GO) annotation as being associated with eye lens structure. Transcripts for a gene of the same family as the human TIN (tubulointerstitial nephritis antigen-related protein, containing a papain-like protease domain in the carboxy-terminal region and one weak somatomedin B-like domain in the amino-terminal region) were not found in the BF library, while six such transcripts were found in the NBF library. No increase in the expression in any cuticular or extracellular matrix genes was found following the blood meal.

3.2. Cytoskeletal proteins (Table 4A and B)

Six gene products were significantly upregulated 24 h after the blood meal, including tubulin (1.58× increase) and a protein from the microtubule cytoskeleton (14× increase) associated with Golgi organization and biogenesis, which may reflect the increased biosynthetic pathways associated with oogenesis. A gap junction transcript is also upregulated (7 × 0), perhaps reflecting the developing ovarian epithelium or the stretched midgut epithelium. On the other hand, 29 transcripts associated with cytoskeletal proteins were downregulated following the blood meal, mostly coding for muscle proteins, including transcripts coding for several troponins, actins, myosins, and flightin. Although anopheline mosquitoes can take multiple blood meals in a single gonotrophic cycle (Briegel and Horler, 1993), it appears that flying around is not a high priority for BF *An. gambiae*.

3.3. Transcription control and nuclear regulation (Table 5A and B)

Most probably associated with the dramatic metabolic needs of digesting a blood meal and converting it to eggs, 19 transcripts tentatively associated with transcriptional and nuclear regulation are upregulated, and five downregulated, 24 h following a blood meal. Among the

Table 2
Categories of transcripts showing increased (up) or decreased (down) expression following a blood meal

Category	Change[a]	Number of genes	Min[b]	Max[c]
Cuticular proteins	Up	0		
	Down	14	1.86	82
Cytoskeletal	Up	6	1.6	14
	Down	29	1.97	35.5
Transcription control	Up	19	2.8	20
	Down	5	1.45	47
Signal transduction	Up	19	2.57	20
	Down	27	1.31	18
Digestion-related proteins	Up	20	1.86	144
	Down	11	2	22
Intermediate metabolism	Up	44	2.3	114
	Down	64	1.21	23
Protein synthesis machinery	Up	52	1.5	19
	Down	1	6	6
Oogenesis	Up	20	3	878
	Down	0		
Immunity and phenol oxidase cascade	Up	9	4.5	10
	Down	1	6	6
Oxidant and environmental stress	Up	5	3	11
	Down	4	3	5.7
Retroviral message	Up	1	2.7	2.7
	Down	0		
Unknown	Up	29	1.7	26
	Down	47	1.57	25

[a] Indicates significant increase (up) or decrease (down) following a blood meal.
[b] Minimal increase or decrease in expression for genes in category.
[c] Maximal increase or decrease in expression for genes in category.

upregulated are several coding for histones, a methyltransferase, several nuclear proteins such as importin, lamin, and helicases. Among the five downregulated transcripts are two coding for polyubiquitins, which may be associated with gene expression regulation (including one that scored 47×0), and three RNA-binding proteins including a protein similar to the TATA binding protein.

3.4. Signal transduction (Table 6A and B)

The blood meal increased transcription of 17 genes associated with signal transduction pathways, including those associated with hormones and neural reception. These included transcripts associated with cell-cycle control (DNA damage checkpoint, cyclin B, prohibitin), neural tissue differentiation and function (including a ligand-gated ion channel, synaptobrevin-like protein 1, an odorant binding protein, and antiquitin, which is a dehydrogenase associated with hearing), protein phosphorylation pathways (a phosphoserine phosphatase and other proteins associated with GTPases and GTP-binding proteins), hormones, and growth factors (imaginal disc growth factor, possibly a steroid hormone receptor, and insulin-like growth factor binding protein). The adipophilin transcript, a protein associated with lipid particles, is included in this category because it is associated with adipose tissue differentiation. The transcript associated with the human polyposis locus protein is also increased; its function is unknown. A transcript coding for a long (320 amino acid residues) trypsin-like protein is highly expressed in the BF library and is included in this signal transduction category because long trypsins are usually associated with tissue growth and differentiation (as indicated by the GO match) or with activating serine protease pathways such as clotting or the phenol oxidase cascade described below (Christophides et al., 2002). Note that this trypsin transcript may actually be involved in oogenesis, a category discussed below that contains several transcripts possibly associated with signal transduction in egg development. Finally, two transcripts having a nitrilase domain associated in other organisms with tissue differentiation and tumor growth have increased representation (~10 fold) in the BF library. They are homologues of the highly conserved genes Vanin (Granjeaud et al., 1999) and the human NIT protein 2 (Pace et al., 2000).

Twenty-seven transcripts associated with signal transduction pathways were significantly reduced 24 h following a blood meal. These included eight transcripts 1.3–9 fold reduced in expression coding for proteins associated with photoreception, which correlates with decreased expression of transcripts associated with eye

Table 3
Messages associated with cuticular and matrix proteins significantly less transcribed following a blood meal

Protein	B[d]	U[e]	Ratio[f]	Comments
agCP8191	22	41	1.9	Endocuticle structural glycoprotein
agCP6034	6	21	3.5	Cuticular protein
agCP5803	3	11	3.7	Cuticular protein
agCP1463	5	28	5.6	Eye lens cuticular protein
agCP6435	0	6	6.0	Cuticular protein
agCP1412	0	6	6.0	Cuticular protein
agCP3308	0	6	6.0	Tubulointerstitial nephritis antigen-related protein precursor—matrix glycoprotein
agCP11052	3	25	8.3	Cuticular protein
agCP8666	2	32	16.0	Cuticular protein
agCP13394	0	22	22.0	Cuticular protein
agCP2272	1	27	27.0	Cuticular protein
agCP3376	1	29	29.0	Eye lens cuticular protein
agCP9080	0	76	76.0	Cuticular protein
agCP11278	1	82	82.0	Probable cuticular protein

[a] Best match to non-redundant protein database of NCBI (supplement only).
[b] Probability of the match occurring by chance (supplement only).
[c] Best match to the Pfam motif (see Materials and methods) (supplement only).
[d] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[e] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[f] When numerator or denominator was zero it was changed to one to calculate ratio.

Table 4
Messages associated with cytoskeletal proteins

Protein	B[a]	U[b]	Ratio [c]	Comments
(A) Significantly more transcribed following a blood meal				
agCP11952	87	55	1.6	Tubulin
agCP4858	10	2	5.0	Phosphatidylethanolamine-binding protein
agCP13252	7	0	7.0	Cytoskeleton-associated protein CKAPI
agCP6262	7	0	7.0	Gap junction protein
agCP13565	17	2	8.5	Radixin/moesin
agCP6096	14	1	14.0	Microtubule cytoskeleton organization and biogenesis
(B) Significantly less transcribed following a blood meal				
agCP15129	36	71	2.0	Actin
agCP7042	7	18	2.6	Microtubule motor
agCP7164	32	84	2.6	Troponin I
agCP5700	3	9	3.0	Troponin C
ebiP9410	9	31	3.4	Non-muscle myosin-II
agCP1394	3	11	3.7	Motor protein
agCP15161	3	11	3.7	Similar to Dm paxillin
agCP3146	9	34	3.8	Troponin C
agCP8921	14	56	4.0	Actin
agCP8924	2	8	4.0	Actin
agCP5034	12	58	4.8	Actin
agCP4365	5	27	5.4	Paramyosin
agCP11379	2	11	5.5	Myosin?
agCP12117	5	28	5.6	Alpha-actinin
agCP12099	42	250	6.0	Myosin ATPase
agCP5679	3	19	6.3	Troponin C
ebiP8408	24	154	6.4	Tropomyosin
agCP12114	58	401	6.9	Myosin ATPase
agCP1186	6	46	7.7	Titin—muscle
agCP8502	16	124	7.8	Myosin heavy chain
agCP14117	0	8	8.0	Myosin?
agCP1597	20	161	8.1	Troponin
agCP9239	1	9	9.0	Fimbrin
agCP15333	0	9	9.0	Tenascin
agCP7785	0	16	16.0	Titin—muscle
agCP12283	3	63	21.0	Actin
agCP5058	9	237	26.3	Actin
agCP5614	0	35	35.0	Troponin C
agCP3724	2	71	35.5	Flightin (muscle protein 27)

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

cuticular proteins mentioned above. Transcripts associated with regulation of intracellular ion levels were reduced after the blood meal, such as two coding for different subunits of the $Na^+ + K^+$-ATPase, for the sarcoplasmic calcium-binding protein, and for another muscle-related protein, tafazzin. The transcript for ENIGMA, a protein associated with receptor-mediated endocytosis, was moderately reduced (2.4 fold), while the transcript coding for a homologue of the vertebrate scavenger receptor class B type I was found nine times in the NBF but not found in the BF library. This receptor in vertebrates is responsible for the uptake of cholesterol from HDL by steroidogenic tissues and could be associated to mosquito organs synthesizing ecdysone-related hormones (Krieger, 1999). Two transcripts coding for proteins of the apoptosis pathway were also reduced. The transcript coding for the enzyme glutamate decarboxylase, which produces the neuromediator γ-amino butyric acid (GABA), was reduced 11 fold after the blood meal, possibly indicating a decreased need of GABAergic neuromediation in BF mosquitoes. Disruption of GABAergic mediation in *Drosophila* led to decreased locomotory activity in these flies (Leal and Neckameyer, 2002). It is thus possible that decreased expression of the glutamate decarboxylase transcript in BF mosquitoes may reflect a decreased need for locomotion, or flight, after the blood meal. Notably, the transcript for the per region controlled protein homologue of *Drosophila* and *Aedes* takeout was not found in the BF library, while 12 such transcripts were found in the NBF library. In *Drosophila*, takeout was found to be a novel molecular link between circadian rhythms and feeding behavior (Sarov-Blat et al., 2000) and may affect feeding behavior patterns in BF mosquitoes. Additionally associated with behavior is the downregulation of

Table 5
Messages associated with transcription and nuclear regulation products

Protein	B[a]	U[b]	Ratio[c]	Comments
(A) Significantly more transcribed following a blood meal				
agCP5560	39	14	2.8	Nucleolus–cytoplasm shuttle phosphoprotein—Nopp140 homologue
agCP9434	11	3	3.7	Cyclin-dependent protein kinase, regulator—mitosis
agCP13790	22	6	3.7	Karyopherin beta 3
agCP10761	19	5	3.8	Histone H1
agCP6986	9	2	4.5	Nucleolar GTPase
agCP9970	43	9	4.8	High mobility group protein 1a—establishment and/or maintenance of chromatin architecture
agCP10116	12	2	6.0	Protein arginine *N*-methyltransferase 1
agCP9811	6	0	6.0	Histone
agCP5959	6	0	6.0	DNA replication factor
agCP8321	6	0	6.0	Stathmin—mitosis regulator
agCP2865	6	0	6.0	Nuclear protein Hcc-1
agCP8365	6	0	6.0	Kruppel homologue 2
agCP10778	19	3	6.3	Histone H1
agCP13868	22	3	7.3	Chromatin-binding protein BJ1
agCP4550	9	1	9.0	ATP dependent RNA helicase
agCP10060	9	0	9.0	Histone H2A.x
agCP8818	13	0	13.0	Importin
agCP10826	40	2	20.0	Histone
agCP11210	7	0	7.0	Lamin—nuclear protein
(B) Significantly less transcribed following a blood meal				
agCP12312	31	45	1.5	Polyubiquitin 1025 aa
agCP14151	31	47	1.5	Ribonucleoprotein—mRNA splicing?
agCP8788	2	10	5.0	Transcription activator C MAF
agCP8674	0	6	6.0	Similar to TATA binding interacting protein
agCP12328	0	47	47.0	Ubiquitin—300 aa

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

two pheromone binding proteins that are decreased 1.4 and 7 × 24 h after the blood meal. Finally, associated with neurophysiology are two transcripts coding for proteins associated with synapses—complexin and a synapse-associated protein—both without representation in the BF library.

3.5. Digestion-related proteins (Table 7A and B)

Following the need to rapidly digest the relatively huge protein meal, 20 transcripts coding for serine proteases and other proteases are increased 24 h after the blood meal, including the trypsin transcripts previously described as being upregulated following a blood meal (Muller et al., 1993) (Table 7A). Seven of these 20 transcripts coding for serine proteases are upregulated from 13 to 144 fold or more. Transcripts coding for structural proteins of the midgut epithelium are also increased, such as that for peritrophin 1 (Shen and Jacobs-Lorena, 1998) and for two mucins, one previously described (Shen et al., 1999), and one novel (agCP1658), which has 70 predicted Ser and Thr mucin-type glycosylation sites (Hansen et al., 1998) and is abundantly transcribed (244 EST following the blood meal). Of notice are also two transcripts coding for dipeptidyl peptidases, similar to angiotensin-converting enzymes (ACE), that are upregulated ~5–4 fold. These ACE enzymes could also be involved, if not in digestion, in peptide hormone conversion. The transcripts for two proteins found in *An. gambiae* salivary glands have increased expression after the blood meal. These two transcripts code for proteins of the SG1 family of salivary proteins, which have no similarities to other known proteins (Francischetti et al., 2002; Lanfrancotti et al., 2002). Both these transcripts derive from genes closely located in the X chromosome of *An. gambiae* and are probably under the same transcriptional control.

Eight transcripts coding for digestive enzymes or structural proteins of the midgut, as well as two transcripts coding for salivary gland proteins, are decreased in expression 24 h following a blood meal (Table 7B). These include transcripts coding for four trypsins previously annotated as early trypsins (Giannoni et al., 2001; Lemos et al., 1996) and one early chymotrypsin (Shen et al., 2000). These serine proteases, as proposed previously, are probably associated with the initial digestion of the blood meal, leading to the activation of the remaining serine proteases described in the previous paragraph. They also have their messages downregulated following the blood meal. Of interest, the predicted *An. gambiae* protein ebiP6721 codes for a protein twice the length of digestive trypsins and containing the sequence for two trypsins, the first of which is 97% identical in amino acid sequence to the previously described trypsin 3 of *An. gambiae* (Muller et al., 1995). Related to sugar meal digestion, transcripts coding for the two midgut maltases (Agm1 and Agm2) (Zheng et al., 1995), both located at the base of chromosome 3, are ~2.5 fold reduced in expression after the blood meal, reflecting the decreased need of a sugar meal during blood digestion. These maltase genes are probably under the same transcriptional control. Two previously undescribed *An. gambiae* proteins similar to *Drosophila* peritrophin A also have reduced expression after the blood meal. These peritrophin genes are located near each other in the base of chromosome 2 and may be under the same transcriptional control. These may represent messages for peritrophin proteins stored in pre-blood meal granules, as proposed before for *An. stephensi* (Berner et al., 1983). Finally, the gene coding for the orthologue of *Aedes aegypti* salivary maltase gene (James et al., 1989)

Table 6
Messages associated with signal transduction products

Protein	B[a]	U[b]	Ratio[c]	Comments
(A) Significantly more transcribed following a blood meal				
agCP5849	18	7	2.6	Neural ion channel?
agCP12509	35	13	2.7	DNA damage checkpoint—mitosis control
agCP11339	19	7	2.7	Neuroendocrine-specific protein
ebiP6959	49	18	2.7	Imaginal disc growth factor
agCP11454	28	9	3.1	Similar to steroid hormone receptor
ebiP3353	14	4	3.5	Polyposis locus protein
agCP9787	11	3	3.7	Rho GDP-dissociation inhibitor 1
agCP10014	20	5	4.0	Phosphoserine phosphatase
agCP13227	39	9	4.3	Adipophilin—adipose differentiation
agCP3606	9	2	4.5	Insulin-like growth factor binding protein complex acid labile chain precursor
agCP9110	15	3	5.0	Antiquitin; dehydrogenase—hearing?
agCP7463	6	0	6.0	Odorant binding protein
agCP7499	6	0	6.0	Synaptobrevin-like protein 1
agCP10268	13	2	6.5	Prohibitin—cell proliferation control
agCP7261	7	0	7.0	Vanin-like pantheteinase
agCP2157	38	3	12.7	G2/mitotic-specific cyclin B
agCP7788	13	0	13.0	NIT protein homologue
agCP14029	14	1	14.0	GTP-binding protein
agCP2731	20	1	20.0	Trypsin-like serine protease—larger than typical digestive enzymes
(B) Significantly less transcribed following a blood meal				
agCP12001	557	731	1.3	Opsin—vision
agCP12026	114	151	1.3	Opsin—vision
agCP5798	85	117	1.4	Arrestin—metharodopsin inactivation
agCP8119	119	168	1.4	Arrestin—metharodopsin inactivation
agCP11484	34	48	1.4	Pheromone binding protein
agCP12420	184	276	1.5	Opsin—vision
agCP15579	13	29	2.2	Antigen 5-related—trypsin inhibitor
agCP1909	19	43	2.3	Photoreceptor membrane-associa—ion channel
agCP12752	7	16	2.3	Apoptosis-regulating basic protein
agCP10937	6	14	2.3	Alpha2-macroglobulin
agCP7676	24	57	2.4	ENIGMA PROTEIN—receptor mediated endocytosis
agCP1846	11	28	2.5	Na^+/K^+ ATPase alpha subunit
agCP11004	26	77	3.0	Sarcoplasmic calcium-binding protein
agCP12526	8	25	3.1	Opsin—vision
agCP6208	31	102	3.3	Ca^{2+}-transporting ATPase—sarcoplasmic reticulum
agCP1515	3	12	4.0	Death associated protein
agCP4884	2	8	4.0	ATPase inhibitor—chaperone regulator
agCP8810	4	16	4.0	Sodium/potassium-transporting ATPase
agCP3311	2	10	5.0	Tafazzin—muscle contraction and development
agCP2386	0	6	6.0	Synapse associated protein
ebiP3863	0	6	6.0	Complexin
agCP11481	0	7	7.0	Pheromone binding protein
agCP13936	1	9	9.0	Cellular retinaldehyde-binding protein—vision
agCP4329	0	9	9.0	Scavenger receptor—HDL receptor—steroidogenesis
agCP1489	1	11	11.0	Glutamate decarboxylase
agCP7122	0	12	12.0	Takeout—per region circadian controlled protein
agCP7677	0	18	18.0	LIM PROTEIN—protein kinase C binding

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

(located at the tip of chromosome 2) had zero transcripts found after the blood meal (12 transcripts found in the NBF library), while another transcript previously found in the salivary gland and coding for protein gSG2 of unknown function (Lanfrancotti et al., 2002) was ~2.4 fold reduced in expression. The observed downregulation of the salivary gland maltase transcript is in agreement with the previously measured temporal rate of

Table 7
Messages associated with digestive proteins

Protein	B[a]	U[b]	Ratio [c]	Comments
(A) Significantly more transcribed following a blood meal				
Gut proteins				
agCP3409	52	28	1.9	Peritrophin 1
agCP11264	269	84	3.2	ANTRYP1 trypsin
agCP1658	244	73	3.3	Mucin—70 *O*-glycosylation sites
agCP12050	19	5	3.8	Mucin
agCP8603	19	4	4.8	Peptidyl-dipeptidase A
agCP3123	183	34	5.4	Chymotrypsin 2 precursor
agCP2202	40	7	5.7	Digestive cysteine proteinase 1
agCP8538	16	2	8.0	Beta-hexosaminidase
ebiP5694	63	7	9.0	Chymotrypsin 1 precursor
agCP14039	13	1	13.0	Angiotensin-converting enzyme
agCP5517	83	5	16.6	Late trypsin
agCP15006	18	0	18.0	Serine protease SP24D precursor
ebiP7547	26	0	26.0	Chymotrypsin 1
agCP3610	73	1	73.0	Trypsin precursor
agCP6269	85	0	85.0	Microvilli protein G12 precursor
agCP5701	86	0	86.0	Distal intestinal serine protease
agCP10888	144	1	144.0	Trypsin 2 precursor
Salivary gland proteins				
agCP13443	17	4	4.3	Salivary protein SG1 family
agCP13537	8	0	8.0	Salivary gsg1b protein
(B) Significantly less transcribed following a blood meal				
Gut proteins				
agCP11302	59	118	2.0	Trypsin 4 precursor
ebiP6721	21	42	2.0	Trypsin 3 precursor—2 trypsins
ebiP8952	14	36	2.6	Maltase-like protein Agm2
agCP9414	13	35	2.7	Maltase-like protein Agm1
agCP12012	10	32	3.2	Serine proteinase
agCP11182	4	15	3.8	Trypsin 5 precursor
agCP15518	1	12	12.0	Peritrophin A
agCP2637	0	22	22.0	Peritrophin A
Salivary gland proteins				
agCP6166	5	12	2.4	Salivary gsg2 salivary protein
agCP12790	0	10	10.0	Salivary maltase

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

resynthesis of the enzyme following the blood meal in *Aedes* (Marinotti et al., 1990).

3.6. Intermediary metabolism (Table 8A and B)

The transcripts coding for 39 enzymes and five transporters probably associated with intermediary metabolism were upregulated 24 h following the blood meal (Table 8A). These indicated increased expression of genes coding for nine enzymes involved in gluconeogenesis, seven in lipid synthesis, nine in purine metabolism, and six lysosomal hydrolases including the highly transcribed cathepsin B previously described in the fat body of mosquitoes (Cho and Raikhel, 1992). Additionally, four transcripts are associated with intracellular degradation of proteins, including two coding for ubiquitin-conjugating enzymes, one for the 19S proteasomal protease, and one for a cytosolic aminopeptidase. One transcript coding for the folate metabolism enzyme dihydrofolate reductase is also increased following the blood meal. Finally, the gene coding for the NADP-dependent isocitrate dehydrogenase is ~5 fold more expressed in the BF library. It is to be noticed that several of the transcripts in this group are also related to the excretion of NH_3, as indicated in Table 8A, including a 9× increase in the enzyme glutamine synthetase I.

On the other hand, genes coding for 64 enzymes and 1 sulfate transporter were significantly less expressed 24 h following a blood meal (Table 8B). Most of these were associated with energy production metabolism, including those coding for 28 enzymes associated with oxidative phosphorylation, nine associated with the glycolytic pathway, and eight associated with the tricarboxylic acid (TCA) cycle. The enzyme adenylate kinase (myokinase), listed as acting on purine metabolism, is an abundant muscle enzyme that plays a role in the synthesis of ATP from ADP and is thus also important for energy metabolism. Furthermore, the genes coding for two enzymes associated with glycogen degradation are significantly less expressed 24 h following the blood meal, as are those coding for one enzyme (triacylglycerol lipase) and two lipoproteins associated with lipid catabolism and transport. Two genes coding for glutamate–ammonia ligases are ~10 fold reduced. Located near each other in chromosome 2, they are probably both regulated under a common transcriptional system. It is interesting to note that the enzyme glutamine synthetase I, which catalyzes a similar reaction, is 10× increased after a blood meal, as indicated above. Also related to glutamate metabolism and ammonia excretion is the gene coding for the enzyme glutamate synthase, with seven EST found previous to, but no transcripts found after, the blood meal.

Finally, genes coding for three enzymes related to glycerolypid metabolism were 3–3.5 fold reduced in their expression 24 h after the blood meal; another three genes associated with detoxication or hormone metabolism were also significantly less expressed after a blood meal. Among these is one coding for a glutathione transferase (12 fold less expressed after a blood meal), contrasting with the increased expression of a similar gene that had a 3.7 fold expression increase after the blood meal (Table 8A). The gene coding for the enzyme dimeric dihydrodiol dehydrogenase had 23 transcripts in the NBF library but none in the BF library. The product of this gene could be associated with steroid hormone metabolism, as is that coding for a P450 enzyme that had 3 fold reduced expression after the blood meal.

Table 8
Messages associated with intermediate metabolism enzymes

Protein	B[a]	U[b]	Ratio [c]	Comments	Metabolic role	E.C. number
(A) Significantly more transcribed following a blood meal						
agCP1576	8	0	8.0	Dihydropteridine reductase	Folate biosynthesis	ec:1.6.99.7
agCP13392	23	10	2.3	Tyrosine aminotransferase	Gluconeogenesis	ec:2.6.1.5
agCP6237	20	8	2.5	3-hydroxyisobutyrate dehydrogenase— mitochondrion	Gluconeogenesis	ec:1.1.1.31
agCP8491	10	1	10.0	Aspartate aminotransferase	Gluconeogenesis	ec:2.6.1.1
agCP13053	29	8	3.6	Methionine adenosyltransferase	Gluconeogenesis	ec:2.5.1.6
agCP14750	20	4	5.0	Glycine *N*-methyltransferase—ser/thr/gly metabolism	Gluconeogenesis	ec:2.1.1.20
agCP5867	24	8	3.0	Tryptophan 5-monooxygenase	Gluconeogenesis—5-HT synthesis	ec:1.14.16.4
agCP4671	11	3	3.7	Glutathione transferase	Gluconeogenesis—detoxication	ec:2.5.1.18
agCP8877	22	5	4.4	Short chain 3-hydroxyacyl-CoA dehydrogenase	Gluconeogenesis—lipid metabolism	ec:1.1.1.36
agCP4738	18	2	9.0	Glutamine synthetase I	Gluconeogenesis—NH_3 excretion	ec:6.3.1.2
agCP8141	12	2	6.0	3,2-*trans*-enoyl-CoA isomerase, mitochondrial precursor	Lipid metabolism	ec:5.3.3.8
ebiP3013	8	0	8.0	Short chain dehydrogenase	Lipid metabolism?	
agCP11068	13	4	3.3	Sphingomyelin phosphodiesterase	Lipid synthesis	ec:3.1.4.12
agCP1726	7	0	7.0	Enoyl-CoA hydratas	Lipid synthesis (path 2)	ec:4.2.1.17
agCP3486	25	7	3.6	Acetyl-CoA C-acyltransferase—mitochondrion	Lipid synthesis (path 2)	ec:2.3.1.16
agCP9560	25	6	4.2	Thiolase	Lipid synthesis (path 2)	ec:2.3.1.9
agCP9862	23	4	5.8	Enoyl-CoA hydratas	Lipid synthesis (path 2)	ec:4.2.1.17
agCP14034	12	0	12.0	Cathepsin B-like cysteine protein	Lysosomal enzyme	ec:3.4.22.1
agCP15371	7	0	7.0	Alpha mannosidase	Lysosomal enzyme	ec:3.2.1.24
agCP1623	16	2	8.0	Acid phosphatase	Lysosomal enzyme	ec:3.1.3.2
agCP14019	114	0	114.0	Cathepsin B	Lysosomal protease	ec:3.4.22.1
agCP14242	31	11	2.8	Lysosomal aspartic protease precursor	Lysosomal protease	ec:3.4.23.5
agCP9609	25	10	2.5	Cathepsin L	Lysosomal protease	ec:3.4.22.15
agCP14692	12	1	12.0	Ubiquitin-protein ligase	Protein degradation	ec:6.3.2.19
agCP15048	11	1	11.0	Ubiquitin-conjugating enzyme	Protein degradation	ec:6.3.2.19
agCP3559	9	0	9.0	Ubiquitin-conjugating enzyme	Protein degradation	ec:6.3.2.19
agCP3758	9	2	4.5	19 S proteasome endopeptidase	Protein degradation	ec:3.4.25.1
ebiP5638	6	0	6.0	Cytosol aminopeptidase	Protein degradation	ec:3.4.11.1
agCP12750	10	2	5.0	Phosphoribosylformylglycinamidine synthase	Purine metabolism	ec:6.3.5.3
agCP13240	50	17	2.9	Phosphoribosylaminoimidazole carboxylase	Purine metabolism	ec:1.17.4.1
agCP3347	12	2	6.0	Ribonucleoside-diphosphate reductase	Purine metabolism	ec:4.1.1.21
agCP3814	60	16	3.8	Nucleoside diphosphate kinase	Purine metabolism	ec:1.17.4.1
agCP6049	27	11	2.5	Purine nucleoside phosphorylase	Purine metabolism	ec:2.4.2.1
agCP6855	11	3	3.7	IMP dehydrogenase—GMP biosynthesis	Purine metabolism	ec:1.1.1.205
agCP7922	11	2	5.5	5′-nucleotidase—not salivary	Purine metabolism	ec:3.1.3.5
agCP8413	6	0	6.0	Similar to phosphoribosylamine-glycine ligase	Purine metabolism	ec:6.3.4.13
agCP8817	9	0	9.0	GMP synthase [glutamine-hydrolyzing]	Purine metabolism—NH_3 excretion	ec:6.3.5.2
ebiP3967	14	2	7.0	Tricarboxylate carrier	Transporter	

(*continued on next page*)

Table 8 (*continued*)

Protein	B[a]	U[b]	Ratio [c]	Comments	Metabolic role	E.C. number
agCP2956	26	5	5.2	Isocitrate dehydrogenase [NADP]	TCA pathway	ec:1.1.1.42
agCP10032	7	0	7.0	Glucose transporter?	Transporter	
agCP14204	6	0	6.0	Probable cation transporter	Transporter	
agCP4602	8	0	8.0	Tyrosine transporter	Transporter	
agCP6220	7	0	7.0	Organic cation transporter	Transporter	
ebiP9622	6	0	6.0	Transporter	Transporter	
(B) Significantly less transcribed following a blood meal						
agCP10713	18	221	12.3	Glutathione transferase	Detoxication	ec:2.5.1.18
agCP4955	0	23	23	Dimeric dihydrodiol dehydrogenase	Detoxication	ec:1.3.1.20
ebiP2093	3	9	3.0	Cytochrome P450	Detoxication—hormone metabolism?	
agCP11342	4	14	3.5	2-hydroxyacylsphingosine 1-beta-galactosyltransferase	Glycerolipid metabolism	ec:2.4.1.45
ebiP7095	4	14	3.5	UDP glycosyltransferase	Glycerolipid metabolism	ec:2.4.1.45
agCP14056	13	54	4.2	Glycerol-3-phosphate dehydrogenase	Glycerolipid metabolism—glycolysis	ec:1.1.1.8
agCP10991	20	35	1.8	Glycogen phosphorylase	Glycogen degradation	ec:2.4.1.1
agCP10643	0	6	6.0	1,4-alpha-glucan branching enzyme	Glycogen metabolism	ec:2.4.1.18
agCP1743	74	118	1.6	Fructose-bisphosphate aldolase	Glycolysis	ec:4.1.2.13
agCP8020	13	26	2.0	Sugar porter—glucose transporter	Glycolysis	
agCP15339	11	23	2.1	Phosphoglycerate kinase	Glycolysis	ec:2.7.2.3
agCP10714	6	14	2.3	Pyruvate dehydrogenase E1 component beta subunit	Glycolysis	ec:1.2.4.1
agCP11165	7	18	2.6	Acylphosphatase, muscle type isozyme	Glycolysis	ec:3.6.1.7
agCP12096	8	22	2.8	Triose-phosphate isomerase	Glycolysis	ec:5.3.1.1
agCP3353	3	13	4.3	Phosphofructokinase	Glycolysis	ec:2.7.1.11
agCP13974	0	6	6.0	Phosphoglycerate mutase	Glycolysis	ec:5.4.2.1
agCP5003	1	12	12.0	Alcohol dehydrogenase	Glycolysis—lipid metabolism	ec:1.1.1.1
agCP1513	4	12	3.0	Triacylglycerol lipase	Lipid metabolism	ec:3.1.1.3
agCP9688	3	10	3.3	Sphingomyelin phosphodiesterase	Lipid synthesis	ec:3.1.4.12
agCP9701	3	20	6.7	Low density lipoprotein receptor	Lipid transport	
agCP9711	1	18	18.0	Low density lipoprotein receptor	Lipid transport	
agCP12411	7	22	3.1	Lysosomal Pro-X carboxypeptidase	Lysosomal carboxypeptidase	ec:3.4.16.2
agCP6407	0	7	7.0	Glutamate synthase	NH_3 metabolism	ec:1.4.1.14
agCP15538	0	9	9.0	Glutamate–ammonia ligase	NH_3 metabolism	Glutamate ammonia ligase
agCP10119	1	10	10.0	Glutamate–ammonia ligase	NH_3 metabolism	Glutamate ammonia ligase
agCP3941	145	175	1.2	ADP, ATP carrier protein	Oxidative phosphorylation	
agCP15237	31	44	1.4	Cytochrome *c* oxidase polypeptide IV	Oxidative phosphorylation	
agCP6905	28	41	1.5	Proton-transporting ATP synthase complex	Oxidative phosphorylation	ec:3.6.3.14
agCP15298	29	43	1.5	Cytochrome *c* oxidase polypeptide Vb, mitochondrial precursor	Oxidative phosphorylation	ec:1.9.3.1

Table 8 (*continued*)

Protein	B[a]	U[b]	Ratio [c]	Comments	Metabolic role	E.C. number
agCP6088	43	64	1.5	Ubiquinol-cytochrome c reductase	Oxidative phosphorylation	ec:1.10.2.2
agCP9526	69	103	1.5	ATP synthase beta subunit	Oxidative phosphorylation	ec:3.6.3.14
agCP7944	30	45	1.5	Hydrogen-transporting two-sector ATPase	Oxidative phosphorylation	ec:3.6.3.14
agCP9085	23	35	1.5	Cytochrome *c* oxidase polypeptide VIA	Oxidative phosphorylation	ec:1.9.3.1
agCP11416	50	77	1.5	ATP synthase gamma subunit	Oxidative phosphorylation	ec:3.6.3.14
agCP6168	52	81	1.6	Arginine kinase	Oxidative phosphorylation	ec:2.7.3.3
agCP13255	14	24	1.7	NADH-ubiquinone oxidoreductase 20 kDa subunit	Oxidative phosphorylation	ec:1.6.5.3
agCP11195	18	31	1.7	ATP synthase delta chain, mitochondrial precursor	Oxidative phosphorylation	ec:3.6.3.14
agCP3454	32	57	1.8	Cytochrome *c* reductase	Oxidative phosphorylation	ec:1.9.99.1
agCP13526	121	220	1.8	ATP synthase lipid-binding	Oxidative phosphorylation	ec:3.6.3.14
agCP7933	12	22	1.8	Phosphate carrier protein—mitochondrial	Oxidative phosphorylation	
agCP15122	69	127	1.8	ATP synthase alpha chain	Oxidative phosphorylation	ec:3.6.3.14
agCP10759	10	19	1.9	NADH dehydrogenase	Oxidative phosphorylation	ec:1.6.5.3
agCP9843	17	36	2.1	Cytochrome Bc1 Complex	Oxidative phosphorylation	ec:1.10.2.2
agCP1427	161	345	2.1	ADP/ATP translocase	Oxidative phosphorylation	
agCP8218	7	16	2.3	NADH-ubiquinone oxidoreductase B22 subunit	Oxidative phosphorylation	ec:1.6.5.3
agCP11479	5	12	2.4	NADH dehydrogenase	Oxidative phosphorylation	ec:1.6.5.3
agCP14772	12	32	2.7	Cytochrome Bc1 Complex	Oxidative phosphorylation	ec:1.10.2.2
agCP6349	7	20	2.9	NADH-ubiquinone oxidoreductase	Oxidative phosphorylation	ec:1.6.5.3
agCP14558	7	22	3.1	Cytochrome *c* oxidase	Oxidative phosphorylation	ec:1.9.3.1
agCP11425	10	33	3.3	Cytochrome *c* oxidase	Oxidative phosphorylation	ec:1.9.3.1
agCP8449	3	12	4.0	NADH-ubiquinone oxidoreductase	Oxidative phosphorylation	ec:1.6.5.3
agCP3870	2	9	4.5	Cytochrome *c* oxidase polypeptide	Oxidative phosphorylation	ec:1.9.3.1
agCP3306	11	51	4.6	Glycerol-3-phosphate dehydrogenase	Oxidative phosphorylation	ec:1.1.1.8
agCP10264	5	22	4.4	Adenylate kinase-1	Purine metabolism—ATP synthesis from ADP	ec:2.7.4.3
agCP6358	3	9	3.0	Xanthine dehydrogenase—peroxisome	Purine metabolism— urate synthesis	ec:1.1.1.204
agCP11153	35	51	1.5	Aconitate hydratase—mitochondrion	TCA cycle	ec:4.2.1.3
agCP2799	27	40	1.5	Succinate-CoA ligase	TCA cycle	ec:6.2.1.5
agCP14738	18	31	1.7	NAD+-isocitrate dehydrogenase	TCA cycle	ec:1.1.1.41
agCP9307	20	36	1.8	Citrate synthase	TCA cycle	ec:4.1.3.7
agCP12505	31	56	1.8	Malate dehydrogenase	TCA cycle	ec:1.1.1.37
agCP4430	20	37	1.9	Pyruvate kinase	TCA cycle	ec:2.7.1.40
agCP1123	18	45	2.5	NAD+-isocitrate dehydrogenase	TCA cycle	ec:1.1.1.41
agCP3730	7	24	3.4	Succinate dehydrogenase	TCA cycle	ec:1.3.99.1
agCP1290	0	7	7.0	Sulfate porter	Transporter	

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

3.7. Protein synthesis (Table 9A and B)

Twenty-four hours following the blood meal, 53 genes coding for products associated with the protein synthesis machinery, with protein folding and modification, and with protein export/secretion were significantly increased in expression. These 53 genes contrast with a single decrease in gene expression for this category following the blood meal, namely a gene coding for a lipoate-protein ligase for which no transcripts were found after the blood meal but six were found previously. The changes in expression for these genes following a blood meal must reflect the intense biosynthetic activity of the fat body, as well as of the midgut and ovaries, following the blood meal.

3.8. Oogenesis-associated proteins (Table 10)

Twenty-two genes coding for proteins probably associated with oogenesis were found to be significantly more expressed 24 h after a blood meal. These included genes coding for three vitellogenins, a major constituent of the yolk, including one coding for a product very similar to the protein named vitellogenin 1 (deposited in GenBank by Patricia Romans, unpublished), for which there were 878 copies in the BF versus no copies in the NBF library (or ~2% of all transcripts found in this library). The two other vitellogenin transcripts code for different vitellogenins similar, but not identical, to the *An. gambiae* vitellogenin 2 protein. One gene coding for a vitelline membrane protein is similarly highly expressed after the blood meal, as is the vitellogenin receptor gene. These data are in agreement with the previously known increase of vitellogenin gene expression in mosquitoes following a blood meal (Kokoza et al., 2001).

Several genes associated with maternal effects on oogenesis were found to have increased expression 24 h after the blood meal, including the homologues of *Drosophila* genes *exuperantia*, *oskar*, *SUMO*, and *fusilli*, involved in dorsal/ventral axis determination, and two other genes coding for proteins weakly similar to the *Drosophila cup* and *notch* proteins. A gene coding for a helicase involved in pole cell determination was also included in this group, as was a gene coding for a lysosomal carboxypeptidase similar to the vitellogenic carboxypeptidase of humans and *Aedes*. This mosquito enzyme was shown to be increased in expression following a blood meal (Deitsch and Raikhel, 1993; Snigirevskaya et al., 1997). Several genes coding for proteins associated with lipid transport are also increased in expression, including one coding for a apolipophorin III homologue (Smith et al., 1994) not previously described in mosquitoes. These gene products could be associated with lipoprotein transport to the oocytes. In accordance with these results, lipophorin in the mosquito *Ae. aegypti* was shown before to be a yolk protein precursor and to have increased message and protein expression peaking at 18 h post-blood meal (Sun et al., 2000). Finally, a gene coding for a class B serpin (similar to ovalbumin) was found to have increased expression, as was one gene coding for a probable extracellular matrix protein.

3.9. Messages associated with immunity and melanization pathways (Table 11A and B)

The phenol oxidase pathway is an important component of invertebrate immunity (Christophides et al., 2002; Soderhall and Cerenius, 1998) and egg-shell melanization (Huang et al., 2001). In this pathway, tyrosine derivatives are oxidized by phenoloxidases or peroxidases to quinones, resulting in crosslinking of the nearby proteins to form a varnish-like coat over the egg shell, or parasites. *An. gambiae* prophenoloxidase message nearly identical to agCP1154 was shown before to be expressed in all life stages, but to have increased expression in eggs (Lee et al., 1998). This enzyme message was not found increased in our data set, but several other messages coding for enzymes in this pathway were found increased 24 h after the blood meal. The messages for tyrosine-related enzymes tyrosine hydroxylase, 4-phenylpyruvate dioxygenase, and aromatic-L-amino acid decarboxylase were 4.5–10 fold increased 24 h after the blood meal. Messages for two serine protease activators of phenol oxidases were also found increased, one activator of the prophenoloxidase, and another for the monophenol monooxygenase activator. Finally, the messages for the terminal enzymes monophenol monooxygenase, laccase, and ovoperoxidase were also increased. Outside the scope of the phenol oxidase pathway, seven EST coding for a protein with a peptidoglycan recognition motif was found in the BF, but none in the NBF library.

Six EST coding for a C-type lectin were found in the NBF library, but none were found in the BF library. C-type lectins may be involved in immunity but also can be associated with other cellular processes (Braakman, 2001; Vasta et al., 1999). A gene coding for α-2 macroglobulin, reported in Table 6B, was also less expressed 24 h after the blood meal. Although proteins from this family are normally rather unspecific protease inhibitors, their role in regulating invertebrate immunity-related protease cascades has been reported (Christophides et al., 2002).

3.10. Messages associated with oxidant and environmental stress (Table 12A and B)

Twenty-four hours after the blood meal, two genes coding for proteins similar to thioredoxins and two coding for a thioredoxin peroxidase and a thioredoxin reductase are expressed more than in unfed mosquitoes. These four gene products could be involved in the pro-

Table 9
Messages associated with the protein synthetic machinery

Protein	B[a]	U[b]	Ratio[c]	Comments
(A) Significantly more transcribed following a blood meal				
agCP6680	165	110	1.5	Ribosomal protein L23a
agCP7766	123	79	1.6	Ribosomal protein S3a
ebiP5182	118	74	1.6	Ribosomal protein L5
agCP1729	117	72	1.6	Ribosomal protein
agCP12518	88	54	1.6	Ribosomal protein S3
agCP1665	116	69	1.7	TCTP protein
agCP14911	64	38	1.7	40S ribosomal protein S9
agCP3905	278	163	1.7	Elongation factor-1alpha
agCP9554	89	51	1.7	Ribosomal protein
agCP13883	114	65	1.8	Ribosomal protein S2
agCP6876	58	33	1.8	60S ribosomal protein L10A
agCP2030	53	29	1.8	Ribosomal L15
agCP1535	44	24	1.8	Ribosomal protein l36e
agCP8523	43	22	2.0	Cyclophilin-33
agCP10200	31	15	2.1	Ribosomal protein S28
ebiP7687	34	16	2.1	Heat shock protein 82
agCP7923	129	56	2.3	60S acidic ribosomal protein
agCP2380	26	11	2.4	Translation elongation factor
agCP3862	20	8	2.5	Translation initiation factor
agCP8746	43	17	2.5	40S ribosomal protein S11
agCP4425	20	7	2.9	Splicing factor—nucleus
agCP5405	15	5	3.0	Heat shock 105kD—chaperone
agCP4869	18	6	3.0	RNA helicase—mRNA splicing
agCP2527	44	14	3.1	Calreticulin
agCP13946	40	12	3.3	RNA binding protein
agCP15469	14	4	3.5	Peptidyl-prolyl *cis–trans* isomerase
agCP13238	11	3	3.7	Eukaryotic translation initiation factor 4B
agCP7767	11	3	3.7	Gal transporter?
agCP14768	26	7	3.7	Splicing factor
agCP14562	12	3	4.0	T-complex protein 1, delta subunit—chaperone
agCP15106	20	5	4.0	Methionine aminopeptidase
agCP12796	17	4	4.3	HSP90—chaperone
agCP4695	17	4	4.3	Translocon transporter
agCP8697	13	3	4.3	KDEL receptor—Golgi to ER transport
agCP15347	13	3	4.3	Translocon-associtaed protein
ebiP3404	9	2	4.5	HPBRII-4 MRNA—mRNA processing
agCP5037	10	2	5.0	Protein translocation complex beta
agCP1844	11	2	5.5	T-complex protein 1, eta subunit—chaperone
agCP14807	11	2	5.5	DNop5 protein—rRNA processing
agCP3947	35	6	5.8	Protein disulfide-isomerase
agCP1793	12	2	6.0	Chaperonin ATPase—protein folding
agCP5562	6	0	6.0	HSP-chaperone
agCP9046	6	0	6.0	Mitochondrial ribosomal protein
agCP14694	7	0	7.0	Signal recognition particle
agCP7941	16	2	8.0	Translation initiation factor
agCP6626	16	2	8.0	Signal sequence receptor
agCP12480	24	3	8.0	Translocon-associated prot
agCP9550	9	0	9.0	mRNA binding protein
agCP5962	9	0	9.0	Possible RNA binding
agCP1688	20	2	10.0	Poly(A)-binding protein
agCP10835	12	0	12.0	Ribosomal protein
agCP8848	19	0	19.0	Splicing factor
(B) Significantly less transcribed following a blood meal				
agCP9684	0	6	6.0	Lipoate-protein ligase

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

Table 10
Messages associated with oogenesis significantly more transcribed following a blood meal

Protein	B[a]	U[b]	Ratio[c]	Comments
agCP2941	30	10	3.0	Apolipophorin-III
agCP15206	18	5	3.6	Pole cell determination—ATP dependent helicase
agCP12201	12	3	4.0	Serpin clade B (ovalbumin type)
ebiP1101	10	2	5.0	Fatty acid-binding protein, musc
ebiP938	31	6	5.2	Fatty acid-binding protein
ebiP9198	6	0	6.0	SUMO protein dorsal/ventral axis determination
agCP1111	7	0	7.0	Fatty acid-binding domain
agCP9337	7	0	7.0	Fusilli, enhancer of cactus—dorsal/ventral pattern determination
agCP12460	92	12	7.7	Apolipophorin precursor
agCP15510	16	2	8.0	Extracellular matrix dentin
agCP15254	11	0	11.0	Weak similarity to *Dm* cup protein
agCP7740	24	2	12.0	Maternal effect protein oskar
agCP13194	12	0	12.0	Vitellogenin receptor
ebiP9426	26	2	13.0	Vitellogenic carboxypeptidase
agCP8969	16	0	16.0	Weakly similar to Notch growth factor—neurogenesis
agCP2354	24	0	24.0	vitellogenin
agCP2542	73	0	73.0	vitellogenin
agCP3927	74	1	74.0	Maternal exuperantia protein
agCP15454	314	0	314.0	Vitelline membrane protein homologue
agCP2518	878	0	878.0	Vitellogenin

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

Table 11
Messages associated with phenol oxidation cascade, and immunity products

Protein	B[a]	U[b]	Ratio[c]	Comments
(A) Significantly more transcribed following a blood meal				
agCP6671	18	4	4.5	Tyrosine hydroxylase
agCP2753	35	6	5.8	4-hydroxyphenylpyruvate dioxygenase
agCP14256	6	0	6.0	Monophenol monooxygenase activator
agCP12017	7	0	7.0	Peptidoglycan recognition
agCP9913	15	2	7.5	Prophenoloxidase activation enzyme
agCP2084	9	0	9.0	Monophenol monooxygenase
agCP4851	10	1	10.0	Aromatic-L-amino acid decarboxylase
agCP15439	10	0	10.0	Laccase
agCP9211	43	3	14.3	Ovoperoxidase
(B) Significantly less transcribed following a blood meal				
agCP6406	0	6	6.0	C type lectin

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

tection from damage by oxidant radicals, as is the gene coding for the mitochondrial Mn superoxide dismutase, which also has increased expression in the BF library.

The genes coding for three heat-shock proteins were significantly less expressed 24 h after the blood meal, as was the gene coding for protein-methionine-S-oxide reductase, an enzyme restoring the oxidant damage to methionine.

3.11. *Messages coding for proteins of unknown function (Table 13A and B)*

Twenty-nine genes expressing proteins of unknown functions had increased expression 24 h following a blood meal, while 47 were significantly reduced. In this category of gene products with an unknown function are included two genes, where 196 and 26 transcripts were

Table 12
Messages associated with oxidant and environmental stress

Protein	B[a]	U[b]	Ratio[c]	Comments
(A) Significantly more transcribed following a blood meal				
agCP13557	15	5	3.0	Thioredoxin reductase (NADPH)
agCP11759	20	5	4.0	Superoxide dismutase
agCP2356	10	0	10.0	Similar to *Mus* spermatid-specific thioredoxin
agCP1990	53	5	10.6	Thioredoxin peroxidase
agCP14330	11	1	11.0	Thioredoxin domain
(B) Significantly less transcribed following a blood meal				
agCP3435	3	9	3.0	HSP20
agCP4263	2	10	5.0	HSP-20
agCP3837	2	11	5.5	Heat shock protein 40—chaperone
agCP9440	3	17	5.7	Protein-methionine-S-oxide reductase

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

found for each in the BF, but none in the NBF library. These transcripts are probably associated with oogenesis, but their function is unknown.

3.12. Retroviral message more expressed following a blood meal

Transcripts coding for protein agCP2262 were 2.7 fold more abundant 24 h following the blood meal (35 versus 13 transcripts in each library). This protein is probably of retroviral origin, being similar to *Drosophila blastopia*. It was not filtered out during annotation of the *An. gambiae* proteome, which attempted to remove retroviral and transposable element sequences from the published proteome (Holt et al., 2002). It is included here because it indicates the activation of such elements by the blood meal, perhaps because they are located near genes with increased expression following a blood meal.

3.13. Abundant messages equally transcribed in both libraries

Finally, Table 13 (Supplement only) shows 37 proteins for which messages were found abundantly and not significantly different in both libraries (50 or more in each of the two libraries). These exemplify abundant constitutive proteins consisting primarily of ribosomal proteins and elongation factors, as well as two ferritins and one actin. The gene promoters for these proteins may be candidates for transforming *An. gambiae* when a constitutive protein expression is targeted, with the understanding that mRNA levels are a result of both gene transcription and the half-life of the message. Accordingly, messages with high mRNA copy number may reflect decreased turnover and not increased expression.

4. Final remarks

The contrasts of mRNA expression between the two cDNA libraries discussed in this paper determined, as indicated before (Holt et al., 2002), both expected and unexpected results. As anticipated, many genes associated with blood digestion, fat body metabolism, protein synthesis, and oogenesis were highly transcribed following the blood meal. Somewhat unexpected were the downregulation, following the blood meal, of mitochondrial (Table 1) and nuclear transcripts associated with mitochondrial metabolism such as those involved on oxidative phosphorylation and the TCA cycle (Table 8B). Together with the decrease in muscle proteins such as myosin, actin, troponins (Table 4B), and other muscle-related transcripts involved in signal transduction (Table 6B, taffazin, cation transport ATPases, calcium-binding protein), these data could be unified to solely indicate less expression of messages associated with striated muscles. The flight muscle alone comprises about 10% of the insect weight and contains mitochondria representing ~40% of the muscle volume (Wigglesworth, 1972). The hungry mosquito has to engage in flight to obtain the blood meal. After the blood meal, the decreased investment in the muscle portfolio of messages may accordingly represent redirection of resources into biosynthetic fat body and ovaries, perhaps best represented by downregulation of NAD- and upregulation of NADP-dependent isocitrate dehydrogenase following the blood meal. In the mitochondrion, one NADH generates three molecules of ATP essential as energy for muscle activity, while NADPH is needed as reducing power in cytoplasmic biosynthetic reactions. Finally, notice that these changes are not of an absolute nature: many of the changes in transcript expression have been of a moderate (less than 2 fold) level.

Also of interest were the reductions, 24 h following the blood meal, of several transcripts associated with

Table 13
Messages of unknown function

Protein	B[a]	U[b]	Ratio[c]	Comments
(A) Significantly more transcribed following a blood meal				
agCP14591	73	43	1.7	
agCP4228	45	24	1.9	
agCP11978	27	11	2.5	
agCP13115	21	8	2.6	
agCP7207	27	9	3.0	
agCP4903	22	7	3.1	
agCP9871	20	6	3.3	
agCP6677	17	5	3.4	
agCP13453	209	57	3.7	
agCP11163	24	6	4.0	
agCP4801	22	5	4.4	Conserved protein
agCP13081	15	3	5.0	Conserved protein
agCP3460	15	3	5.0	
agCP3611	12	2	6.0	Esterase?
agCP9140	12	2	6.0	
agCP9305	6	0	6.0	
agCP7907	6	0	6.0	Conserved protein
agCP8081	6	0	6.0	
agCP13715	6	0	6.0	
agCP8526	19	3	6.3	Conserved protein
agCP3046	7	0	7.0	
agCP6373	7	0	7.0	
agCP3840	7	0	7.0	
agCP13114	44	6	7.3	
agCP4174	9	0	9.0	Similar to Dm gi:7300374
agCP13045	11	1	11.0	Conserved—selenium protein? Oxidant stress?
agCP11468	14	1	14.0	
agCP15442	26	0	26.0	NtR gene product—ion channel? oogenesis related?
agCP12846	196	0	196.0	Low complexity—Gly rich—oogenesis related?
(B) Significantly less transcribed following a blood meal				
agCP2471	60	94	1.6	
agCP7485	31	50	1.6	
agCP14622	69	112	1.6	
agCP1451	23	38	1.7	
agCP4171	23	38	1.7	
agCP4376	11	20	1.8	
agCP14504	12	22	1.8	
agCP7943	26	50	1.9	
agCP4568	12	25	2.1	
agCP7276	9	19	2.1	
agCP13599	13	28	2.2	
agCP11471	12	26	2.2	Similar to *Dm* Kisir protein—conserved
agCP10836	7	16	2.3	
agCP13884	6	14	2.3	
agCP5057	5	12	2.4	
agCP2153	6	15	2.5	
agCP10259	9	23	2.6	Similar to human involucrin—low complexity protein—similar to peptide hormone—sperm displacement
agCP12023	4	11	2.8	
agCP1671	5	14	2.8	
agCP13264	7	20	2.9	
agCP8353	3	9	3.0	
agCP11347	3	9	3.0	Similar to *Tenebrio* melanization-related protein—similar to vertebrate vitellogenins
agCP7210	6	19	3.2	Hypoxia induced gene

(*continued on next page*)

Table 13 (*continued*)

Protein	B[a]	U[b]	Ratio[c]	Comments
ebiP7514	4	14	3.5	
agCP8216	3	11	3.7	
agCP4865	4	16	4.0	Similar to immune induced proteins
agCP5825	3	12	4.0	
agCP3513	3	12	4.0	
agCP7535	2	8	4.0	Hydrolase?
agCP3728	2	8	4.0	
agCP1522	2	9	4.5	
agCP3134	2	9	4.5	Conserved protein
agCP6092	3	14	4.7	EN protein binding—gene/engrailed nuclear homeoprotein-regulated gene
agCP1342	2	10	5.0	
agCP5474	0	6	6.0	
ebiP2368	0	6	6.0	Conserved protein
agCP8208	2	14	7.0	
ebiP2957	0	7	7.0	Similar to *Cryptosporidium* oocyst wall protein
agCP1171	4	31	7.8	Similar to ecdysteroid regulated 16 kDa protein
agCP2758	1	10	10.0	
agCP13905	1	10	10.0	
agCP6199	1	11	11.0	
agCP8151	1	13	13.0	
agCP6333	1	15	15.0	
agCP11080	1	17	17.0	
agCP5664	0	20	20.0	
agCP15562	1	25	25.0	

[a] Number of ESTs from BF mosquitoes matching protein sequence identified in Protein field.
[b] Number of ESTs from NBF mosquitoes matching protein sequence identified in Sequence name field.
[c] When numerator or denominator was zero it was changed to one to calculate ratio.

behaviour. These included a moderate loss of transcripts associated with vision, including cuticular proteins associated with the eye lens (Table 3), and several proteins associated with the vision process and pheromone-binding proteins (Table 6B). A gene associated with circadian control of feeding behavior, homologue of *Drosophila* takeout, was also reduced, as was the transcript coding for the enzyme that synthesizes GABA, a mediator associated with insect activity and feeding behavior (Leal and Neckameyer, 2002) (Table 6B). Together, these data indicate, to the extent that transcription actually reflects translation, that BF *An. gambiae* mosquitoes may be in a state of less interest in their surroundings than starved mosquitoes, a pattern that fits with the decreased muscle investment described above.

The postgenomic era of medical entomology, starting with publication of the *An. gambiae* genome, represents a revolution in our way of thinking. This revolution brings massive amounts of data. Although much of the analytical work is descriptive, the output is very rich in identifying many new gene products that may be associated with fundamental aspects of *An. gambiae* biology. In particular, it should be taken in consideration that the work described here reflects overall changes in the whole adult female mosquito transcriptome following a blood meal. Identification of the tissue or tissues responsible for the observed changes remains to be done. Furthermore, equivalent changes in gene expression in two different organs, but in opposite direction, would cancel each other and would not be detected; or, a large change in one transcript in one particular organ could obscure an opposite smaller change in another organ. Ultimately, each major mosquito organ should be tested, possibly with the use of DNA arrays containing the whole transcriptome set, for the identification of genes regulated by the blood meal, or any other physiological change. Gene disruption techniques can determine the impact of each gene product on metabolism and behavior, so that ultimately, the biology of *An. gambiae* will be understood.

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